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THE ORIGIN OF CENTRAL AND PERIPHERAL P-HYDROXY-PHENYLACETIC ACID IN MAN AND RATS

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ABSTRACT

The effects of a number of monoamine oxidase, two types of dopadecarboxylase inhibitors and neomycin on the production of p-hydroxyphenylacetic acid (PHPA) and catecholamine metabolites were evaluated in an attempt to determine the origin of central and peripheral PHPA in rats. Acute intragastric (I.G.) administration of pargyline as well as chronic I.G. carbidopa, alpha-methyldopa (dopadecarboxylase inhibitors) and neomycin treatments failed to reduce PHPA concentration in the brain and urine, suggesting minor roles of gut flora and endogenously produced p-tyramine in the overall body production of PHPA. Neomycin reduced p-Ty and increased PHPA excretion. Catecholamine metabolites, phenylethylamine and p-tyramine (p-Ty) excretions were altered according to the expected mode of action of the drugs employed. Paradoxically, carbidopa (like alpha-methyldopa) significantly reduced hypothalamic norepinephrine and its metabolism, suggesting a central influence of carbidopa. Chronic administration of three types of monoamine oxidase (MAO) inhibitors, pargyline, clorgyline and deprenyl failed to reduced urine and brain PHPA. These drugs produced changes in phenylethylamine and catecholamine metabolite's excretion and brain content that are consistent with effective inhibition of either or both MAO type A and B. By a process of elimination, it is concluded that while most body p-Ty is derived from p-tyrosine decarboxylation, most central and peripheral PHPA in rats and possibly man originate from p-tyrosine transamination to p-hydroxyphenylpyruvic acid followed by decarboxylation to PHPA. This conclusion was confirmed by demonstrating that the administration of p-hydroxyphenylpyruvic

acid significantly elevated the excretion of PHPA as well as p-hydroxyphenyllactic acid and homogentisic acid. Blockade of p-tyrosine decarboxylation by carbidopa was also demonstrated to elevate the excretion of deuterated PHPA derived from administered deuterated p-tyrosine, thus adding support to the above conclusion. The contribution of p-tyramine metabolism towards total body output of PHPA is less than 30%. The role of the gut flora in the production of phenylethylamine and catecholamine metabolites was also concluded to be minor. A number of new observations related to p-Ty, PHPA and PEA were made. p-Ty was found to be almost completely excreted in rat in the conjugated form. In contrast most urine PHPA (around 80%) and PEA (70-90%) are excreted unconjugated. It is concluded that although p-Ty may be an important biogenic amine, its metabolism and turnover rate, unfortunately, cannot be assessed from the assay of PHPA.

INTRODUCTION

A number of recent reports on the central and peripheral concentration of p-hydroxyphenylacetic acid (PHPA) suggest a precursor/metabolite relationship between p-tyramine (p-Ty) and PHPA in both man and the experimental animal (Durden and Boulton, 1981, Yu et al., 1982; Boulton et al., 1983). Furthermore, PHPA excretion appears to be abnormally low in aghoraphobic patients and in violent and sexual offenders (Boulton et al., 1983; Davis et al., 1983). There is also a tendency for PHPA concentration in the cerebrospinal fluid to be low in patients with neurological and psychiatric disorders (Kobayashi et al., 1983), a finding that may be of diagnostic importance if confirmed and proven to be consistent. Against this interesting background, however, are the observations of poor correlations between in vivo monoamine oxidase (MAO) inhibition (as determined by the reduction in catecholamine metabolism and increased p-tyramine excretion) and PHPA excretion in man (Karoum et al., 1982; Linnoila et al., 1982). The possibility may, therefore, exist that most urinary PHPA in man is derived from a source other than p-tyramine deamination.

To specifically determine the metabolic origin of PHPA, a number of experiments were conducted to evaluate the contribution of p-Ty metabolism towards central and peripheral PHPA production. As will be shown, and in accordance with human observation, most urine and brain PHPA in the rat does not originate from p-Ty.

MATERIAL AND METHODS

Urine was collected from rats placed in "Econo Metabolic Units" (American Scientific Products, Springfield, Va.). Capillary columns were purchased from Supelco Inc., Pa. Brain parts were dissected according to Glowinski and Iversen, 1966. All chemicals and reagents used were of the highest grades commerically available.

Brain tissues were homogenized in 0.5 ml of 1N HCl containing 50 ng of each of the following deuterated compounds: p-tyramine ($^2H_4$-p-Ty), 3,4-dihydroxyphenylacetic acid (DOPAC) ($^2H_5$-DOPAC), homovanillic acid (HVA) ($^2H_5$-HVA), $^2H_4$-PHPA and phenylethylamine (PEA) ($^2H_9$-PEA). Ten or five μl of the homogenates were pipetted out for protein determination (Lowry et al., 1951). The rest of the homogenates were centrifuged at 15,000 g (Eppendorf 5412 Centrifuged, American Scientific Products) and the clear supernatant stored at -10°C until analyzed.

Urine creatinine was measured by a modification of the original method of Here, 1950.

Unconjugated dopamine, NE, p-Ty and PEA were measured mass fragmentographically as previously reported (Karoum, 1983). A 30 m fused silica capillary column, 0.32 mm i.d. bond coated with SPB-5 (methylphenyl-vinyl-silicone gum) was used for the assay of catecholamines, their metabolites and PHPA. A 30 m fused silica capillary column bond coated with a mixture of SP-2250 (methyl-phenylpronyl silicone gum) SPB-5 and SP 2401 (fluoropropyl silicone) was used for the assay of PEA, m- and p-tyramine.

Total PEA, p- and m-Ty are measured as follows: 50 μl of urine is mixed with 100 ng $^2H_9$-PEA and $^2H_4$-Ty followed by the addition of 100 μl 10 N HCl. The mixture is heated at 100°C for 60 minutes. After hydrolysis, 100 μl of 10 N NaOH is added, mixed and the amines assayed as for free Ty and PEA (Karoum, 1983).

Dopamine metabolites, DOPAC and HVA, p-hydroxyphenyllactic acid (PHPLA), homogentisic acid and PHPA in urine or brain tissues were assayed as previously published (Karoum, 1983).

A model 4000 Finnigan gas chromatograph-quadrupole mass spectrometer (Finnigan Corp., Sunnyvale, California) was used for the mass-fragmentographic measurement of the various amines and metabolites.

Monoamine oxidase inhibitors, pargyline, clorgyline and deprenyl were dissolved in saline and administered intraperitoneally (I.P.) or intragastrically (I.G.). Alpha-methyldopa and carbidopa were admistered I.G. from a saline suspension. The doses and treatment regiments are described in the appropriate Tables.

RESULTS

Preliminary studies on the presence of total (assayed after hydrolysis of urine in 6N HCl and at 100°C for one hour (Martin et al., 1979) and free PHPA concentration in rat urine revealed about 20% of total PHPA is excreted conjugated. Further, intravenous administration of deuterated PHPA ($^2H_9$-PHPA), 10 mg/kg, failed to appear in both the hypothalamus and caudate nucleus after periods ranging from 0 to 2 hours following its administration. It is theretore assumed that peripherally formed PHPA does not efficiently cross the blood brain barrier and that most rat urine PHPA is excreted unconjugated. Furthermore, while over 80% of total p-Ty in urine is conjugated most urine PEA in rats is free (70-90%).

The Role of the Gut Flora in the Production of PHPA and Other Metabolites of Biogenic Amines

To assess the extent of drug inhibition of amino acid decarboxylase and MAO, PEA, p-Ty, NE and DA as well as their metabolites (except that of PEA) were measured in addition to PHPA. To isolate the effects of gut flora metabolism of p-Ty from that of peripheral tissues, PHPA excretion was measured in rats after two weeks chronic neomycin treatment. To further investigate into the contribution of the gut flora to the overall body production of PHPA via p-Ty metabolism, the effects of intragastric (I.G.) administrations of pargyline (MAO inhibitor), carbidopa (peripheral amino acid decarboxylase inhibitor) and alpha-methyldopa (peripheral and central amino acid decarboxylase inhibitor) were evaluated. As shown in Table 1 and 2, neither destruction of the gut flora with neomycin nor inhibition of p-Ty production and metabolism (both intragastrically and within the periphery and the brain) significantly reduced the excretion of PHPA. In contrast to what was expected, neomycin significantly increased PHPA excretion while p-Ty excretion was significantly reduced.

The results in Table 1 also illustrate the effects of the above I.G. treatments on PEA and catecholamine metabolites excretion. As can be deduced from the results MAO and amino acid decarboxylase activities within the body were markedly reduced. Thus, the two amino acid decarboxylase inhibitors employed, significantly reduced PEA excretion. MAO was also markedly inhibited because the excretion of HVA and MHPG were also reduced.

None ot the above treatments changed brain PHPA excretion, Table 2.

TABLE I

THE EFFECTS OF INTRAGASTRIC (I.G.) ACUTE PARGYLINE AND CHRONIC CARBIDOPA, ALPHA-METHYLDOPA AND NEOMYCIN TREATMENTS ON URINE CONCENTRATIONS OF BIOGENIC AMINES AND METABOLITES IN RAT

Description	Total PEA	Total p-Ty	Free PHPA	Free HVA	Total MHPG
Urine					
Controls (μg)	0.314 ± 0.030	16.5 ± 3.7	62.6 ± 10.8	6.6 ± 0.3	5.48 ± 0.50
Pargyline (μg)[a]	3.081 ± 0.275**	41.4 ± 12.8**	44.3 ± 10.8	4.8 ± 0.6*	3.42 ± 0.38**
Carbidopa (μg)[b]	0.169 ± 0.025**	5.92 ± 0.93**	46.0 ± 6.2	5.8 ± 0.8	2.92 ± 0.40**
Alpha-Methyl-dopa (μg)[b]	0.066 ± 0.015**	2.87 ± 0.35**	36.3 ± 1.9**	3.7 ± 0.4*	2.02 ± 0.11**
Neomycin (μg)[c]	0.290 ± 0.042	6.26 ± 1.12**	127.6 ± 38.1*	--	--

[a]Pargyline was administered at a dose of 50 mg/kg and the rats placed in metabolic cages for four hours to collect urine. After four hours the rats were decapitated and their brains remove and analyzed.

[b]Carbidopa, 100 mg/kg, and alpha-methyldopa, 400 mg/kg, were administered I.G. once daily for five days. On the fifth day urines were collected for four hours after the last injection and rats decapitated soon after urine collection. The brains were removed dissected out and analyzed.

[c]Neomycin, 125 mg/kg, I.G. was administered twice daily for two weeks and urine collected overnight after the last treatment. Baseline urines were collected on the same rates before neomycin treatment. All statistical evaluation for the neomycin treated rates were carried out and paired t-test. Neomycin baseline values were comparable to those of the controls and therefore not shown. Five rats were included in each group.

Results are expressed in μg/mg creatinine.

*$p < 0.05$, **$p < 0.001$ compared to the appropriate controls or baselines

TABLE 2

THE EFFECTS OF INTRAGASTRIC (I.G.) ACUTE PARGYLINE AND CHRONIC CARBIDOPA, ALPHA-METHYLDOPA AND NEOMYCIN TREATMENTS ON CAUDATE NUCLEUS CONCENTRATIONS OF BIOGENIC AMINES AND METABOLITES IN RAT

Description	Free PHPA	Free DOPAC	Free HVA
Controls (ng)	0.500 ± 0.0058	8.75 ± 0.44	6.50 ± 0.56
Pargyline (ng)	0.515 ± 0.042	1.18 ± 0.35***	1.56 ± 0.83***
Carbidopa (ng)	0.481 ± 0.036	10.45 ± 0.53	9.12 ± 0.65
Alpha-Methyldopa (ng)	0.451 ± 0.029	7.82 ± 0.39**	5.0 ± 0.49

Brain Results are expressed in ng/mg protein

**$p < 0.005$, ***$p < 0.001$ compared to the appropriate controls or baselines

See Table 1 for explanation of experiment and dosage.

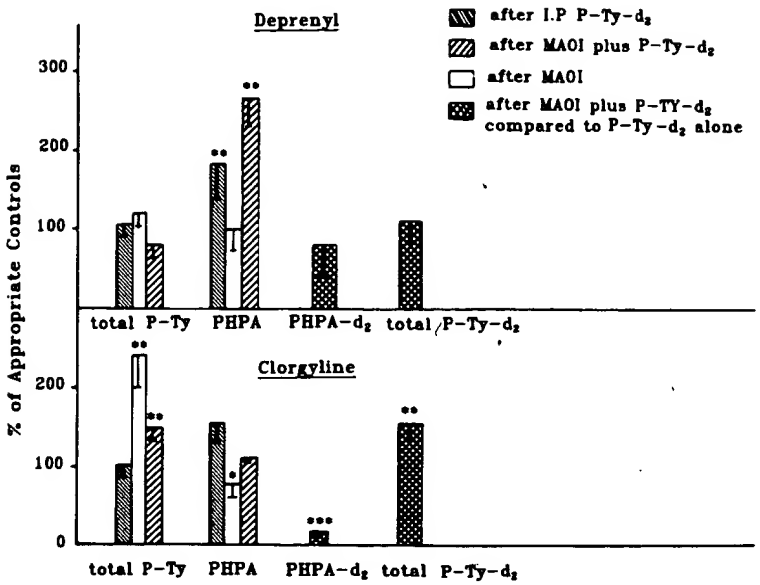

FIGURE 1

Changes in total p-tyramine (p-Ty), p-hydroxyphenylacetic acid (PHPA) and their deuterated isomers excretions in rats challenged with 2 mg deuterated p-Ty (p-Ty-d_2) before and after six days treatments with deprenyl 10 kg, and clorgyline 25 mg/kg, I.P. There MAOIs were administered 12 hours and 1 hour before challenging with p-Ty-d_2.

The percent changes in the non-deuterated compounds are as compared to the appropriate baselines. Those of p-Ty-d_2 and PHPA-d_2 (shown) represent the percent changes as compared to the excretions ot these compounds after 2 mg p-Ty-d_2 challenge before MAOI.

Evaluation of MAO Inhibitions by Clorgyline and Deprenyl and Their Correlations to PHPA Excretion

The results so far presented (Tables 1 and 2) suggest that in vivo inhibition of MAO by pargyline does not significantly reduce PHPA excretion. Since pargyline (at a high dose) is a mixed MAO type A and B inhibitor and since MAO activities within the rat bodies were not measured, the possibility may exist that MAO was not efficiently inhibited, hence the poor reduction in PHPA (Table 1). To assess MAO inhibition with specific reference to p-Ty, the metabolism of I.P. administered $^2H_2$-p-Ty to $^2H_2$-PHPA was evaluated before and after I.P. treatments with clorgyline (a potent MAO type A inhibitor) and deprenyl (an MAO type B inhibitor) (Knoll, 1980). The results are summarized in Figure 1. As shown, clorgyline markedly inhibited $^2H_2$-p-Ty metabolism but reduced PHPA excretion by only about 30%. Deprenyl did not significantly reduce PHPA excretion nor $^2H_2$-p-Ty metabolism. Interestingly, the administration of $^2H_2$-p-Ty significantly increased PHPA excretion, an effect that may be due to stimulation of PHPA production. The results shown in Figure 1 clearly indicate that while p-Ty is efficiently metabolized to PHPA (as represented by $^2H_2$-p-Ty metabolism) most urine PHPA does not apparently originate from p-Ty metabolism. Furthermore, the production of PHPA seems to be enhanced by the availability of free p-Ty in the body. While clorgyline significantly increased both total p-Ty and ($^2H_2$)-p-Ty, deprenyl did not affect total p-Ty nor total ($^2H_2$)-p-Ty excretions. Deprenyl markedly increased total PEA excretion signifying a potent inhibition of MAO type B (results not shown).

The Effects of Chronic MAO Inhibition on PHPA and Other Biogenic Amines Metabolites Excretion and Brain Concentrations

It could be argued that in spite of the relatively high doses of pargyline, clorgyline and deprenyl used, long term inhibition of MAO is needed to affectively reduce PHPA excretion. For this reason, the effects of chronic I.P. administrations of pargyline, clorgyline and deprenyl on the excretion and brain concentrations of a number of amines and/or their metabolites were assessed after two weeks of treatments. The results are summarized in Table 3 and 4. As shown, marked inhibition of NE and DA metabolism by these three MAO inhibitors, was not accompanied by a similar reduction in PHPA in urine or brain. In fact a slight, but significant increase in caudate nucleus PHPA was found after clorgyline. As expected, pargyline and deprenyl but not clorgyline significantly increased free PEA excretion. Based on the observed increase in PEA excretion, the doses of pargyline, clorgyline and deprenyl employed in the present chronic study appear to be within

TABLE 3

THE EFFECTS OF CHRONIC I.P. ADMINISTRATION OF THREE TYPES OF MONOAMINE OXIDASE INHIBITIONS ON URINE EXCRETION OF A NUMBER OF BIOGENIC AMINES AND METABOLITES

Description (unit)	Free PEA	Free p-Ty	Free PHPA	Free DOPAC	Free HVA	Total MHPG
Urine Controls (μg)	0.272 ± 0.029	1.64 ± 0.25	59 ± 8	0.658 ± 0.110	4.1 ± 0.4	5.5 ± 0.4
Pargyline (μg)	2.873 ± 108**	2.44 ± 0.88	58 ± 8	0.424 ± 0.044*	1.9 ± 0.2**	1.8 ± 0.2**
Clorgyline (μg)	0.287 ± 76	2.09 ± 0.33	62 ± 8	0.345 ± 0.049**	1.5 ± 0.1**	0.9 ± 0.1**
Deprenyl (μg)	0.838 ± 116**	0.87 ± 0.16	41 ± 8	0.544 ± 0.053	2.1 ± 0.1**	2.0 ± 0.2**

Pargyline, 7 mg/kg, clorgyline, 6 mg/kg and deprenyl, 5 mg/kg were administered I.P. once a day for two weeks and urine collected for four hours after the last injection. The rats were decapitated four hours after the last injection and the brains analyzed as described in the text. Five rats were included in each experiment.

Results are expressed as mean ± SEM per mg creatinine.

*$p < 0.05$; **$p < 0.005$ by unpaired t-test as compared to the appropriate controls.

TABLE 4

THE EFFECTS OF CHRONIC I.P. ADMINISTRATION OF THREE TYPES OF MONOAMINE OXIDASE INHIBITIONS ON BRAIN CONCENTRATION OF A NUMBER OF BIOGENIC AMINES AND METABOLITES

	Caudate Nucleus			Hypothalamus
Description (unit)	Free PHPA	Free DOPAC	Free HVA	Total MHPG
Caudate Nucleus[c] Controls (ng)	0.570 ± 0.050	9.9 ± 0.70	8.65 ± 0.76	1.48 ± 0.1
Pargyline (ng)	0.588 ± 0.064	1.7 ± 0.23**	2.84 ± 0.45**	1.11 ± 0.05**
Clorgyline (ng)	0.753 ± 0.048**	0.72 ± 0.04**	0.49 ± 0.2**	0.02**
Deprenyl (ng)	0.676 ± 0.034	3.43 ± 0.34**	4.3 ± 0.32**	1.19 ± 0.02

Pargyline, 7 mg/kg, clorgyline, 6 mg/kg and deprenyl, 5 mg/kg were administered I.P. once a day for two weeks and urine collected for four hours after the last injection. The rats were decapitated four hours after the last injection and the brains analyzed as described in the text. Five rats were included in each experiment.

[c]Results are expressed as mean ± SEM per mg protein.

**$p < 0.005$ by unpaired t-test as compared to the appropriate controls.

the appropriate ranges that induce specific MAO type A or type B inhibitions.

Conversion of P-Hydroxyphenylpyruvic Acid to Homogentisic Acid, P-Hydroxyphenyllactic acid (PHPLA) and PHPA

Theoretically, it most of urine and brain PHPA does not arise from the metabolism ot p-tyramine as concluded from the data shown in Tables 1-4, a plausible precursor of PHPA could be p-hydroxyphenylpyruvic acid; a compound reported to be converted in the rabbit to PHPA (Kirberger and Bucher, 1952). This latter phenolic acid is derived from p-tyrosine transamination. p-hydroxyphenylpyruvic acid is known to be efficiently hydroxylated at the ortho (two ring position) position by phenylpyruvic acid oxidase to homogentisic acid followed by ring cleavage by homogentisic acid oxidase to give 4-maleyl-acetoacetic acid (Medes, 1932). However, p-hydroxyphenylpyruvic acid decarboxylation to PHPA has not been well documented in rats nor has the identity of PHPA derived from administered p-hydroxyphenylpyruvic acid been structurally confirmed.

To investigate into the possible conversion ot p-hydroxyphenylpyruvic acid to PHPA, 5 mg of this acid was administered intravenously to five rats and their urines collected for four hours immediately after treatment. The following compounds were measured: PHPA, p-hydroxyphenyllactic acid (PHPLA), homogentisic acid, and p-tyrosine. The results are summarized in Table 5. As shown, about the same amounts of PHPA and PHPLA were formed from p-hydroxyphenylpyruvic acid but the percent increase in PHPLA is 20 fold higher than that found for PHPA. Although the design ot the experiment was not intended to critically trace the metabolism of p-hydroxyphenylphenylpruvic acid, it has nevertheless been established here to be metabolized to PHPA in rats.

Conversion of P-Tyrosine to PHPA

The results in Table 5 together with those reported by Kirberger and Bucher, 1952 regarding the conversion of p-hydroxyphenylpyruvic acid to PHPA can be criticized on account of the chemically unstable nature of p-hydroxyphenylpyruvic acid. Thus one could argue that the elevations observed in PHPA following p-hydroxyphenylpyruvic acid are artifacts resulting from the chemical decomposition within the body of p-hydroxyphenylpyruvic acid to PHPA. Furthermore, administration ot p-tyrosine alone and evaluating the change in PHPA excretion as performed by Kirberger and Bucher, 1952 cannot difterentiate between the contributions arising from p-Ty metabolism and that arising from p-hydroxyphenylpyruvic acid decarboxylation. For these reasons

TABLE 5

METABOLISM OF P-HYDROXYPHENYLPYRUVIC ACID (HPPA) IN RAT[a]

Description	Free PHPA	Free PHPLA	Free Homogentisic Acid	Free p-Tyrosine
Baseline	46.0 ± 5.2	2.58 ± 0.83	None	7.14 ± 1.52
HPPA	70.9 ± 9.6*	27.73 ± 10.0**	111.3 ± 27.4	9.23 ± 0.97
% Change[b]	43 ± 18.4	1204 ± 594	--	48.6 ± 27.8
Absolute change	24.8 ± 11.8	25.20 ± 9.44	111.3 ± 27.4	2.09 ± 1.72

[a]Urine was collected from five rats overnight (baseline). On the following day 5 mg of HPPA was administered I.V. and urine collected immediately for four hours.

[b]The results represent the means ± SEM of % change in each rat.

All results are in μg/mg creatinine (mean ± SEM).

*$p < 0.05$; **$p < 0.005$ by paired t-test as compared to the appropriate baselines.

TABLE 6

THE FORMATION OF $^2H_2$-PHPA FROM $^2H_2$-P-TYROSINE IN RATS BEFORE AND AFTER CARBIDOPA CO-ADMINISTRATION

Description	P-Tyrosine	$^2H_2$-P-Tyrosine	PHPA	$^2H_2$-PHPA
Baseline	129.4 ± 23.3	---	59.3 ± 3.3	---
$^2H_2$-P-Tyrosine[a]	49.1 ± 15.3*	247.0 ± 70.5	38.7 ± 4.5*	5.9 ± 1.0
$^2H_2$-P-Tyrosine[b] plus Carbidopa	86.6 ± 11.7	625.6 ± 58.1*	53.3 ± 5.6	29.7 ± 4.1*

[a]$^2H_2$-p-tyrosine, 5 mg was administered intragestrically twice daily for three days. On the third day and following the last drug administration, urine was collected overnight.

[b]$^2H_2$-p-tyrosine, 5 mg plus carbidopa, 100 mg/kg was administered twice daily for three days. On the third day and following the last treatment, urine was collected overnight.

Five rats were included in each experiment. Results in μg/mg creatinine ± SEM.

*$p < 0.005$ as compared to baseline (non-deuterated compounds) or to $^2H_2$-p-tyrosine (deuterated compounds) by paired t-test.

the conversion of deuterated p-tyrosine to $^2H_2$-PHPA was assessed after three days daily administration of $^2H_2$-p-tyrosine alone and together with 100 mg/kg carbidopa. Co-administration of carbidopa with $^2H_2$-p-tyrosine resulted in about six-fold increase in $^2H_2$-PHPA as compared to when $^2H_2$-p-tyrosine was given alone. These results are summarized in Table 6. Carbidopa administration significantly increased p-tyrosine and $^2H_2$-p-tyrosine excretions. Interestingly, PHPA excretion was significantly reduced after $^2H_2$-p-tyrosine probably as a result of competition with p-tyrosine for the various enzymatic pathways leading to the production of PHPA.

DISCUSSION

Theoretically, there are four feasible sources of PHPA in the body. 1) Dietary PHPA; 2) Deamination of dietary p-Ty by MAO followed by oxidation of the product; 3) Decarboxylation of p-tyrosine (Boulton and Juorio, 1983) to p-tyramine followed by its metabolism as in "2" and finally; 4) Transamination of p-tyrosine to p-hydroxyphenypyruvic acid followed by its decarboxylation to PHPA (Medes, 1932). The 4th source of PHPA may follow the same biochemical sequence as those found in phenylalanine transamination pathway (Curtius et al., 1972; Karoum et al., 1984) and L-DOPA (Calne et al., 1969). The second, third and fourth sources of body PHPA may originate in the gut and/or endogenously within the body. In this context it should be mentioned that decarboxylation of p-tyrosine is not the only source of endogenous p-Ty. Pathways which can lead to the production of p-Ty include p-hydroxylation of phenylethylamine (Boulton et al., 1974; Silkaitis and Mosnaim, 1976) and dehydroxylation of dopa and dopamine (Boulton and Dyck, 1974).

The failure of I.G. administrations of pargyline, neomycin and amino acid decarboxylase inhibitors (Table 1) to alter PHPA urinary excretion strongly suggest a minor role of gut flora p-Ty synthesis or metabolism in the overall body production of PHPA. The marked reductions in PEA, m-Ty and p-Ty excretion observed after carbidopa and alpha-methyldopa also suggest that most of these amines are derived respectively from phenylalanine and p-tyrosine decarboxylation and not from dietary sources (Boulton and Juorio, 1983). The failure of neomycin to reduce PHPA excretion in rats is consistent with several reports in the literature on the effect of gut sterilization on p-tyramine and PHPA excretion (Sandler et al., 1969; Boulton, 1976; DeQuattro and Sjoerdsma, 1976). Furthermore, the significant reduction in total p-Ty and the no change in m-Ty excretion (results not shown) after neomycin are curious but nevertheless in line with a number of recent reports of

reciprocal changes in m- and p-Ty productions after various pharmacological manipulations (Juorio, 1982). Whether this latter observation is coincidental or due to the removal of p-Ty inhibitory effects on m-Ty synthesis is open to future exploration.

Inhibition of p-Ty metabolism by clorgyline (a type A MAO inhibitor) produced a modest but a significant reduction in PHPA excretion, Table 2. This result together with the observed efficient metabolism of deuterated p-Ty to deuterated PHPA clearly demonstrated that while at best only about 30% of total urine PHPA originate from p-Ty, p-Ty itself once formed is rapidly and efficiently metabolized to PHPA. For example, about 40% of the deuterated p-Ty administered was excreted in the form of deuterated PHPA within four hours after treatment (compare results in Table 4).

p-Tyramine was not measured in the brain but its metabolite was estimated after both acute and chronic pargyline (Table 2 and 4) as well as after chronic treatments with two amino acid decarboxylase inhibitors (Table 1), clorgyline and deprenyl (Table 4). None of these drug treatments reduce PHPA concentration in the caudate nucleus. These results appear at first sight inconsistent with reports of decreased mouse caudate nucleus m-and p-hydroxyphenylacetic acid following pargyline administration (McQuade et al., 1981). While species difference may be responsible, it should be noticed that the dose of acute (50 mg/kg) and chronic (7 mg/kg) pargyline used here are considerably lower than the 100 mg/kg employed by these workers. Even at this high dose both m- and p-hydroxyphenylacetic acid concentrations were reduced by less than 30%. Therefore the results reported by McQuade et al. (1981) are in general not very contradictory to those reported here. It should also be emphasized that failure of MAO or amino acid decarboxylase inhibitors to significantly reduce PHPA brain concentrations does not in any way imply that p-Ty is not endogenously produced nor that it is devoid of any physiological function. What these data signify is that, the contribution of p-Ty produced in the periphery and the brain towards total body PHPA production is small compared to other metabolic pathways of tyrosine. In fact it could be argued that the availability of an efficient alternative pathway to p-tyrosine metabolism (as will be discussed later) offers a safe mechanism that prevents excessive production of p-Ty from p-tyrosine. p-Tyrosine administration has been shown to increase striatal concentration of p-ty (Juorio and Boulton, 1982). Further, it is possible that a breakdown in this safety mechanism may be associated with the various disorders that are characterized by abnormal p-Ty production.

Although it is difficult to conclusively eliminate the contribution of dietary PHPA towards PHPA urinary excretion in rats, such a possibility is probably not of major consideration in humans to whom chronic MAO inhibitors treatments produced inconsistent reductions in PHPA excretion (Karoum et al., 1982; Linnoila et al., 1982; additional unpublished results). Further, if most urinary PHPA is derived from the diet, this should not intluence brain PHPA concentration because peripheral PHPA at relatively low concentrations does not efficiently cross the blood brain barrier (McQuade and Juorio, 1983; see Result section). Thus, making the reasonable assumption that dietary PHPA contribution towards peripheral tissue production and ultimately excretion of PHPA is small, the major source of urinary PHPA can therefore be best attributed to p-tyrosine transamination (source 4). For this reason changes in PHPA excretion in rats (after pharmacological manipulations) and by extrapolation in humans, will most likely be associated with abnormal p-tyrosine transamination than with alteration in p-tyramine metabolism. Formation of PHPA from p-hydroxyphenylpyruvic acid is supported by the results in Tables 5 and 6. As shown, p-hydroxyphenylpyruvic acid administration significantly increased PHPA excretion as well as p-hydroxyphenyllactic acid (PHPLA); a stable metabolite of p-hydroxyphenylpyruvic acid (Karoum et al., 1975). PHPA, PHPLA and p-hydroxyphenylpyruvic acid excretions are elevated in both tyrosinemia (increased blood tyrosine) and tyrosyluria (increased urine excretion of both p-tyrosine and its metabolites) (Mede, 1932; Wong et al., 1967; Partington, 1968; Karoum, 1970; Karoum et al., 1975) of the newborn. These two conditions are associated with a transient reduction in p-hydroxyphenylpyruvic acid oxidase in the newborn period of life especially in premature infants (Bloxam et al., 1960). The results presented in Table 5 reflect upon the metabolism of exogenously administered p-hydroxyphenylpyruvic acid and as such may not faithfully mimic the metabolism of the endogenously produced compound. Considering all the results presented (Tables 1-6), the ability of the body to decarboxylate p-hydroxyphenylpyruvic acid to PHPA can be regarded successfully demonstrated.

The results summarized in Tables 1 on the effect of neomycin on PEA excretion can also be used to argue against a major role of the gut flora in the production of PEA in rats this effect is similar to observations in humans (Karoum et al., 1980).

The data presented here reveal a number of interesting characteristics of pargyline, clorgyline and deprenyl. For example, 10 mg/kg deprenyl had very little effect on p-Ty metabolism as compared to clorgyline (Table 4) but like the other two MAO

inhibitors it very affectively reduce NE and DA metabolism both peripherally (as deduced from urine analysis) and centrally (Tables 4). The failure of deprenyl to reduce p-Ty metabolism may be related to the absence ot the so-called "chesse effects" following deprenyl treatment (Elsworth et al., 1978). Further, while pargyline markedly elevated PEA excretion it also shares many similarities with clorgyline. Again all three MAO inhibitors tested produced potent central effects on NE metabolism.

In conclusion, although the origin of most urine PHPA could have been predicted from some old literature (Medes, 1932; Kirberger and Bucher, 1952) these reports did not address certain critical areas such as the structural identities of the compounds measured and the effects pharmacological manipulations have on p-Ty production and metabolism. Furthermore, these old reports did not evaluate the origin of central PHPA. Thus as presented in this communication, a number of pharmacological manipulations were conducted to assess the central and peripheral origin of PHPA in rats. The results ot these experiments suggest that most central and peripheral PHPA does not originate from p-tyramine metabolism. Transamination of p-tyrosine to p-hydroxyphenylpyruvic acid followed by its decarboxylation is probably the major source of PHPA in rats and possibly humans too.

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